

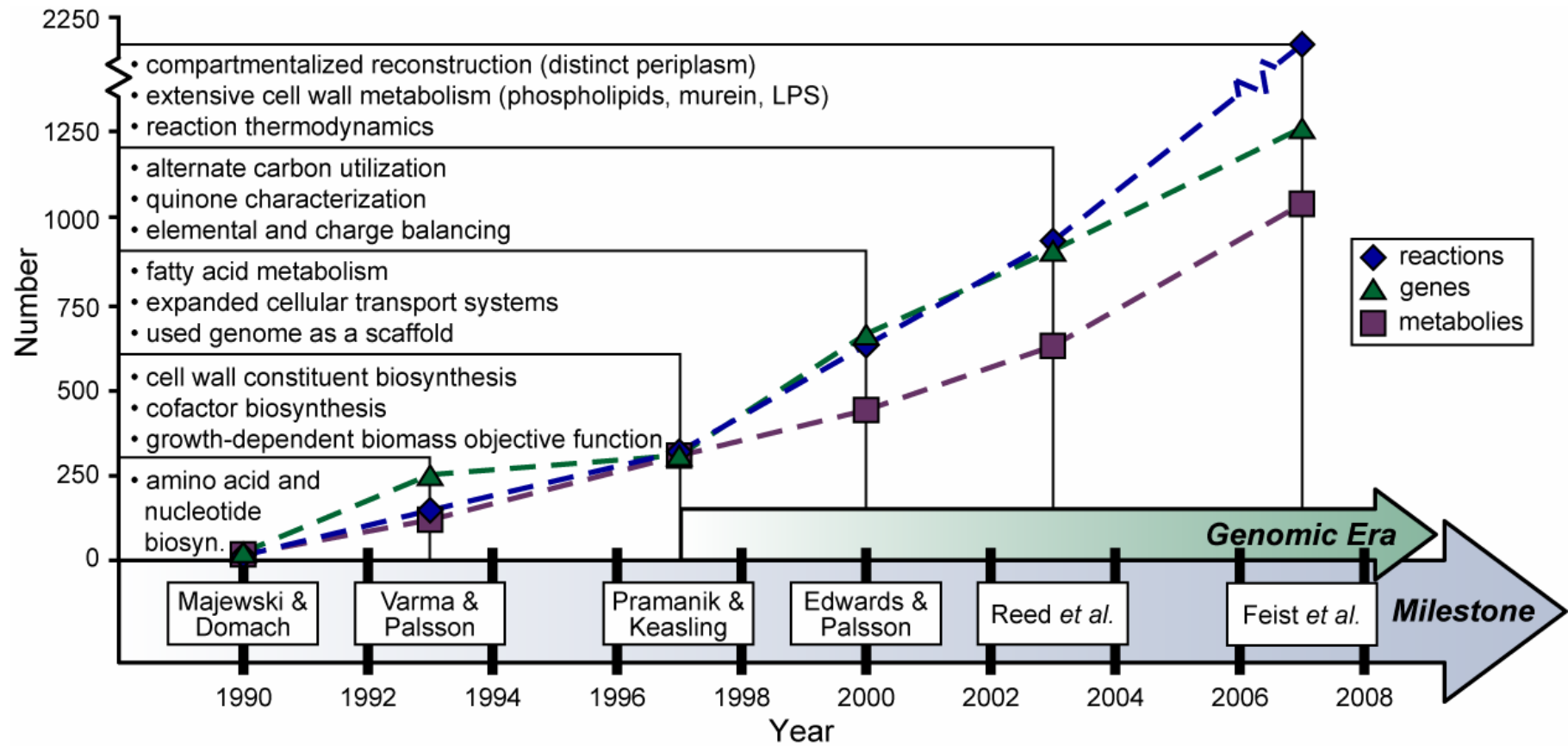
The Challenge of Incorporating Regulatory Effect in Genome-scale Networks

Bernhard Palsson
Bioengineering, UCSD
GtL meeting, Feb 07

Outline

- History of metabolic reconstruction
- Uses of metabolic reconstructions
- Regulation as a constraint-based process
- Measuring TRN components on a g/s
- TRN reconstruction
- Integrated analysis of regulation and metabolism

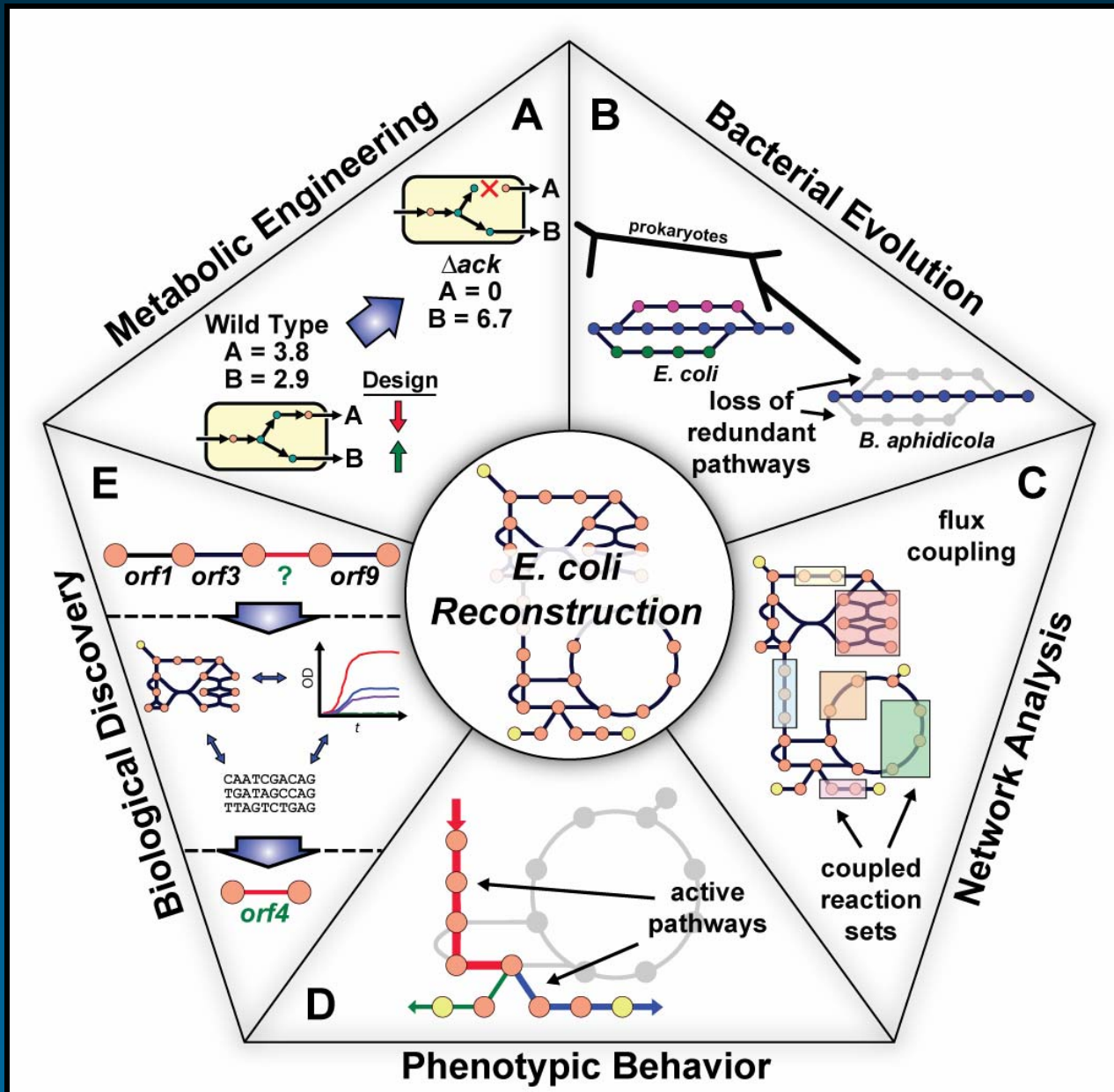
Reconstruction of *E. coli* Metabolism



References:

- R.A. Majewski and M.M. Domach, *Biotechnol Bioeng* **35**, 732 (1990)
- A. Varma, B.W. Boesch, and B.O. Palsson, *Appl Environ Microbiol* **59** (8), 2465 (1993) & *Biotechnol Bioeng* **42** (1), 59 (1993)
- J. Pramanik and J.D. Keasling, *Biotechnol Bioeng* **56** (4), 398 (1997) & **60** (2), 230 (1998)
- J.S. Edwards and B.O. Palsson, *Proc Natl Acad Sci U S A* **97** (10), 5528 (2000)
- J.L. Reed, T.D. Vo, C.H. Schilling et al., *Genome Biology* **4** (9), R54.1 (2003)
- A.M. Feist, C.S. Henry, J.L. Reed et al., *Under Review* (2007)

Uses of the *E. coli* Reconstruction



Metabolic Engineering:

1. *Biotechnol Bioeng* **84**, 647 (2003)
2. *Biotechnol Bioeng* **84**, 887 (2003)
3. *Genome Res* **14**, 2367 (2004)
4. *Metab Eng* **7**, 155 (2005)
5. *Nat Biotechnol* **23**, 612 (2005)
6. *Appl Environ Microbiol* **71**, 7880 (2005)
7. *Metab Eng* **8**, 1 (2006)
8. *Appl Microbiol Biotechnol* **V73**, 887 (2006)
9. *Biotechnol Bioeng* **91**, 643 (2005)

Bacterial Evolution:

10. *Bioinformatics* **21** Suppl 2, ii222 (2005)
11. *Nat Genet* **37**, 1372 (2005)
12. *Nature* **440**, 667 (2006)

Network Analysis:

13. *Bioinformatics* **19**, 1027 (2003)
14. *Genome Res* **14**, 301 (2004)
15. *Nature* **427**, 839 (2004)
16. *Biophys J* **88**, 37 (2005)
17. *PLoS Comput Biol* **1**, e68 (2005)
18. *BMC Bioinformatics* **7**, 111 (2006)
19. *Proc Natl Acad Sci U S A* **102**, 19103 (2005)

Phenotypic Behavior:

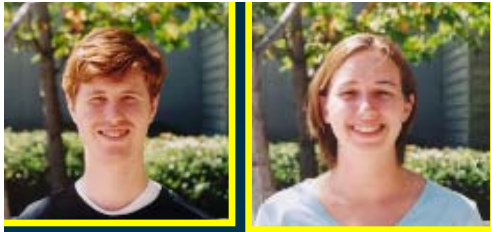
20. *Mol Syst Biol*, Under Review (2007)
21. *Biophys J* **83**, 79 (2002)
22. *Proc Natl Acad Sci U S A* **99**, 15112 (2002)
23. *Bioinformatics* **21**, 2008 (2005)
24. *Proc Natl Acad Sci U S A* **102**, 7695 (2005)
25. *J theor Biol* **237**, 401 (2005)
26. *Biophys J* **90**, 1453 (2006)
27. *BMC Bioinformatics* **7**, 118 (2006)
28. *Mol Syst Biol* **2**, 2006 0034 (2006)
29. *Biophys J* **91**, 2304 (2006)
30. *BMC Bioinformatics* **7**, 512 (2006)
31. *Biophys J* **92**, 1792 (2007)
32. *Nature Genetics* **36**, 1056 (2004)
33. *Nat Biotechnol* **19**, 125 (2001)
34. *Nature* **420**, 186 (2002)

Biological Discovery:

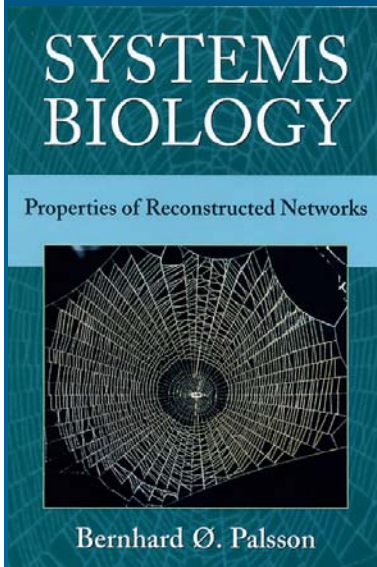
35. *Biophys J* **90**, 2659 (2006)
36. *Genome Biology* **7**, R17 (2006)
37. *BMC Bioinformatics* **7**, 177 (2006)
38. *Proc Natl Acad Sci U S A* **103**, 17480 (2006)
39. *Nature* **429**, 92 (2004)
40. *PLoS Computational Biology* **2**, e72 (2006)

#1 Pathways operate as elements of a network

We can now describe metabolism at a
genome level



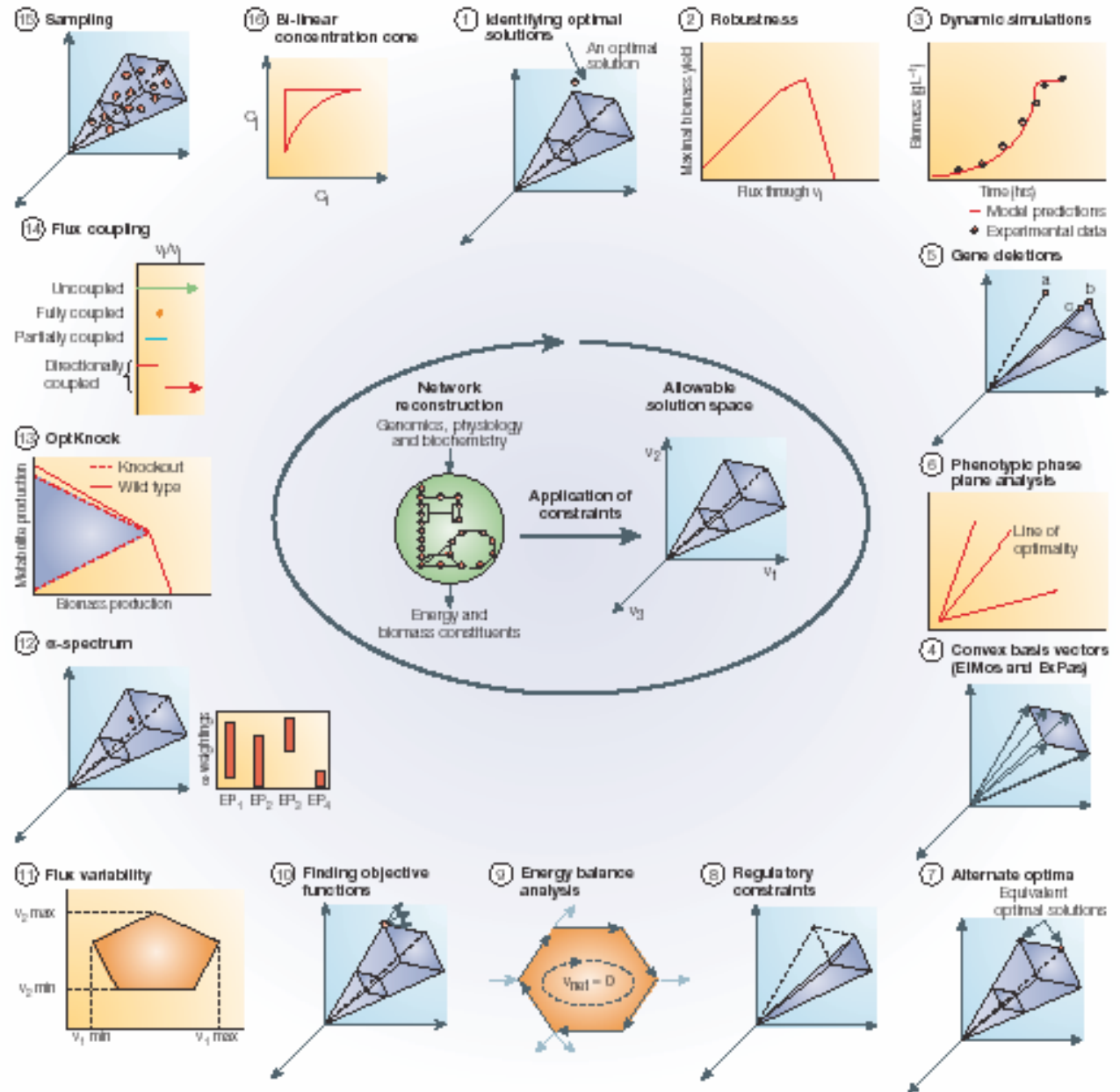
Genome-scale constraint-based modeling: a rapidly growing



San Diego
California

UCSD Extension

Systems Biology Research Group
Price, et al Nat Rev Microbiol. Nov 2004
<http://systemsbiology.ucsd.edu>



Top-down view of regulation

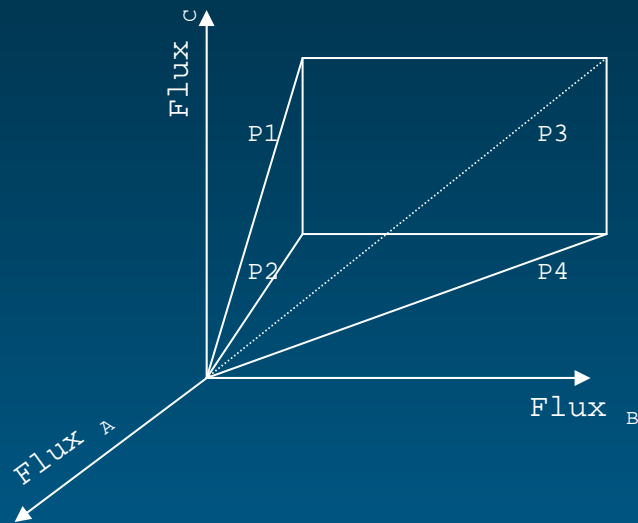
#2: Regulation of expression: shrinking solution space

- Need TRN reconstructed -- now

#3: Regulation of activity: location within a shrunken solution space

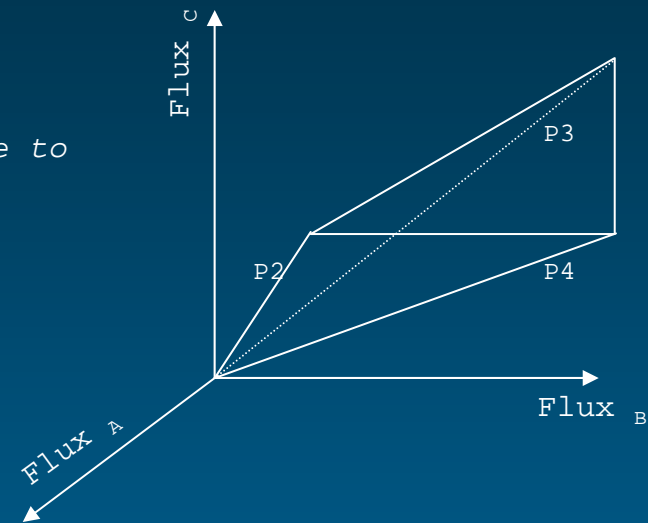
- Sampling -- future

Extreme Pathways and Regulatory Constraints



P1
P2
P3
P4

P1 is not permitted due to regulatory constraints



P1
P2
P3
P4

Consider the entire solution space of a metabolic network, bounded by extreme pathways P1-P4...

One or more of these pathways may not be feasible, depending on the environment and corresponding regulatory effects...

This leads to a reduced solution space bounded by fewer extreme pathways

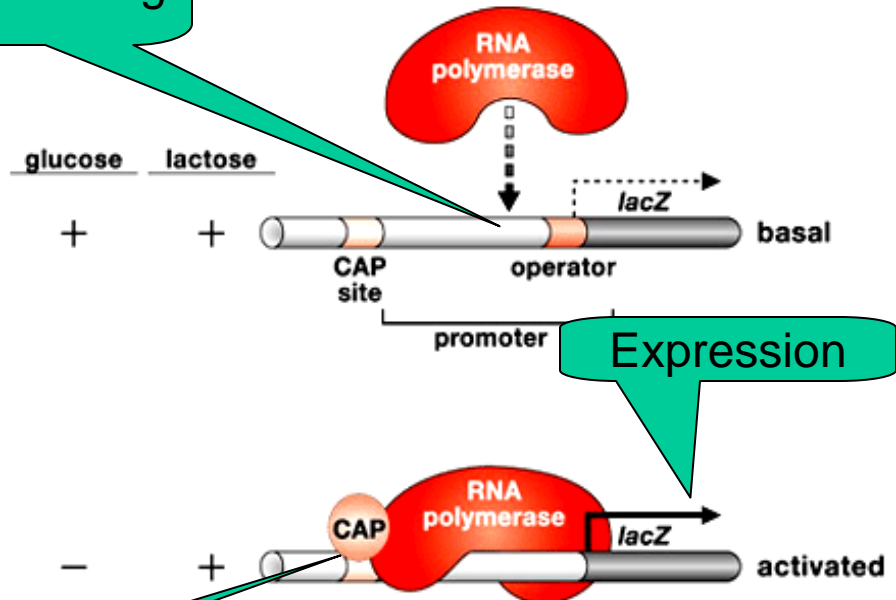
Transcription: RNAP & TFs

RNA polymerase

Transcription factors (TFs)
bind to specific binding sites in
the promoter region of a gene

After binding to DNA, TFs
either enhance or disrupt RNA
polymerase binding to DNA

RNAP binding



TF binding



Drill-down Studies on *E. coli* Transcriptional Sub-networks

Condition	Regulator(s)	Target Functionality	Target Locus
Nitrate	NarL/NarP	Glycolytic Pathways	tpl, pfkA, pf1AB, focA, mdhA, aceAB, aceEF, pykF, eno, pgmA
Nitrate	NarL/NarP	Hydroxylamine Reductase	ybjW
Nitrate	NarL/NarP	Methylglyoxal Synthesis	mgaA
Nitrate	NarL/NarP	Molybdopterin Biosynthesis	moeABCDE, mogAB, moeAB
Nitrate	NarL/NarP	O6 and O4-methylguanine-DNA methyltransferase	ogt
Nitrate	NarL/NarP	Threonine Degradation	kbl, tdh
Oxygen	ArcA/Fnr	Acetate Metabolism	ackA, acnAB, acs, gltA, mdhA, pta
Oxygen	ArcA/Fnr	Fatty Acid Metabolism	fadL, fadD, fadE, fadBA, fadJ, atoSBDA
Oxygen	ArcA	Sialic acid Transport	mgfABC
Oxygen	ArcA/Fnr	Glycerol Metabolism	glcA, ugpABCEQ, glpABC, glpD
Oxygen	ArcA	Glycerol Transport	ugpBAECQ
Oxygen	Fnr	Glycolytic Pathways	pykA, pgmA
Oxygen	ArcA/Fnr	Methionine Degradation	ysaA
Oxygen	ArcA	Nucleotide Metabolism	nudE
Oxygen	Fnr	Peptide Degradation	pepE
Oxygen	ArcA	Potassium Transport	kefB
Oxygen	ArcA/Fnr	α -Ketoglutarate Transport	kgtP
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Acetoacetylbutyrate Synthesis	lmn, lnb
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Alternative DMSO Respiratory Pathway	ynrEFGH
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Aspartate-Asparagine Interconversion	aspA
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Betaine Biosynthesis	betAB1, proP
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Curl (Amyloid) Synthesis	csgABDEFG, cri
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Folic Acid Biosynthesis	folE
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Glucose Degradation	gcd
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Nucleotide Metabolism	udhA
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Oligopeptide Transport	oppABCDF, cstA
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Stationary Phase/ Stress Response	rpoS, cri, yjeE
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Superoxide Response	soxS, raxABCDGE
Oxygen/Nitrate	ArcA/Fnr, NarL/NarP	Thiamin Biosynthesis	thiC

#4: can get the necessary data for
g/s reconstruction of TRN

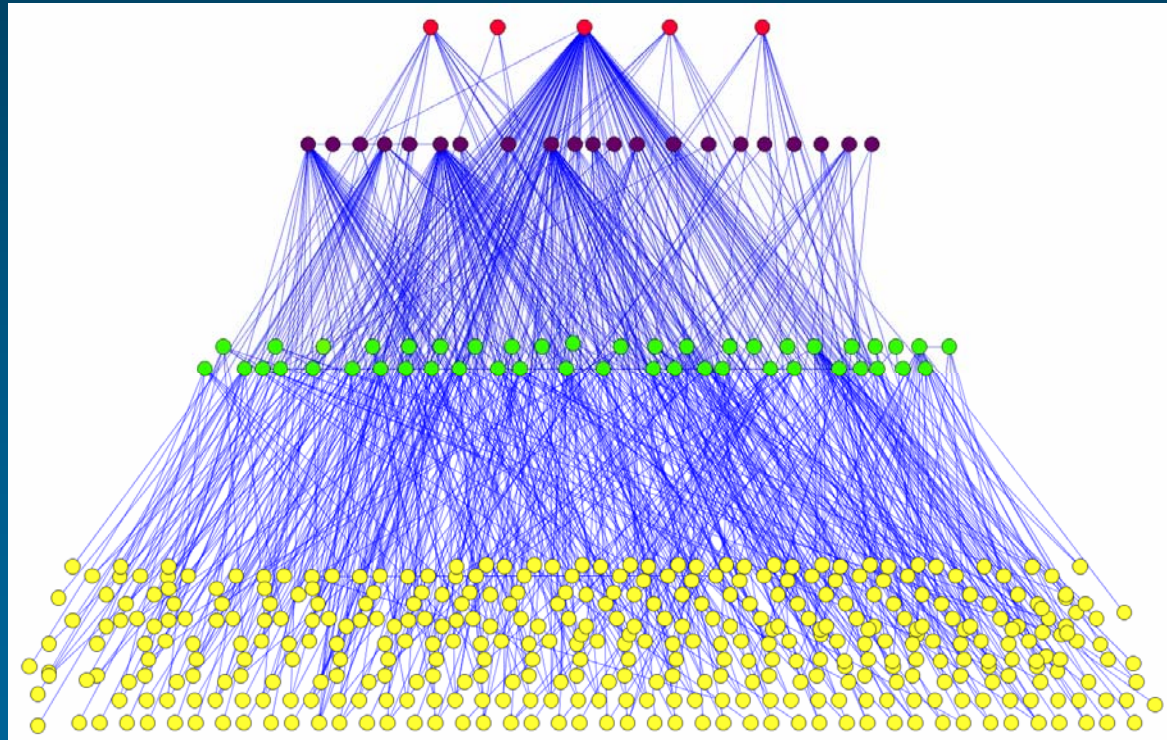
MC1010 – Hierarchy of regulator

global regulators

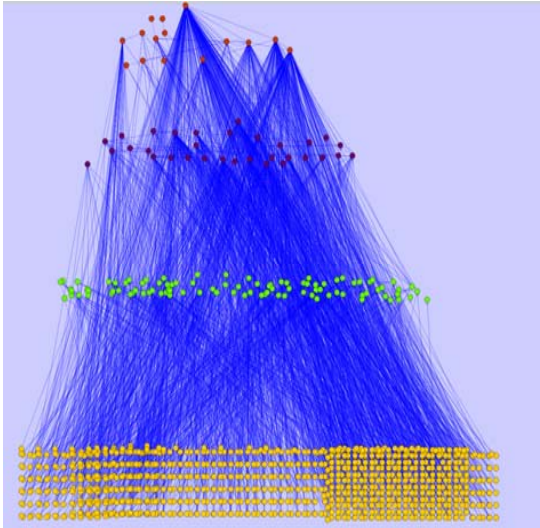
major regulators

minor regulators

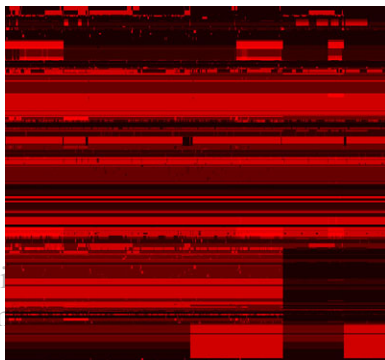
target genes



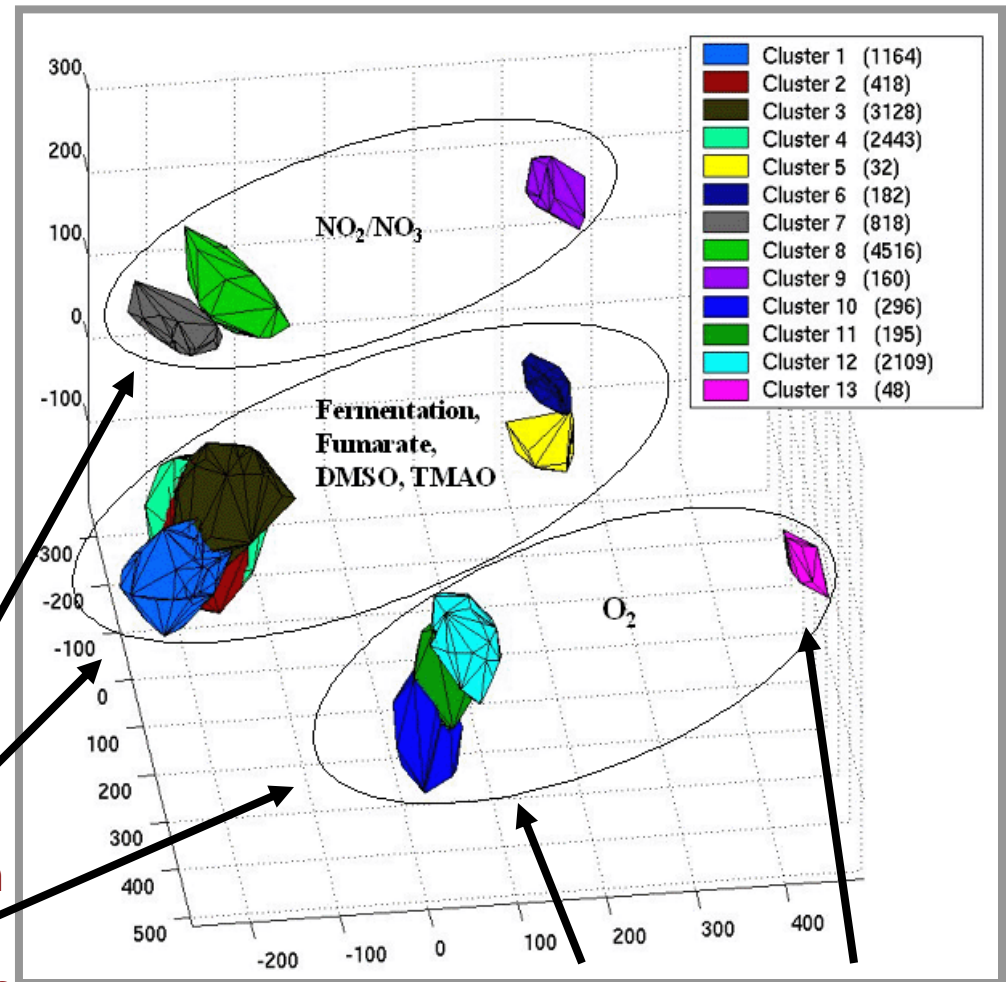
Integrating metabolism and TRN



- Computed Metabolic & Regulatory State in **15,580** distinct minimal growth media



The electron acceptor distinguishes clusters



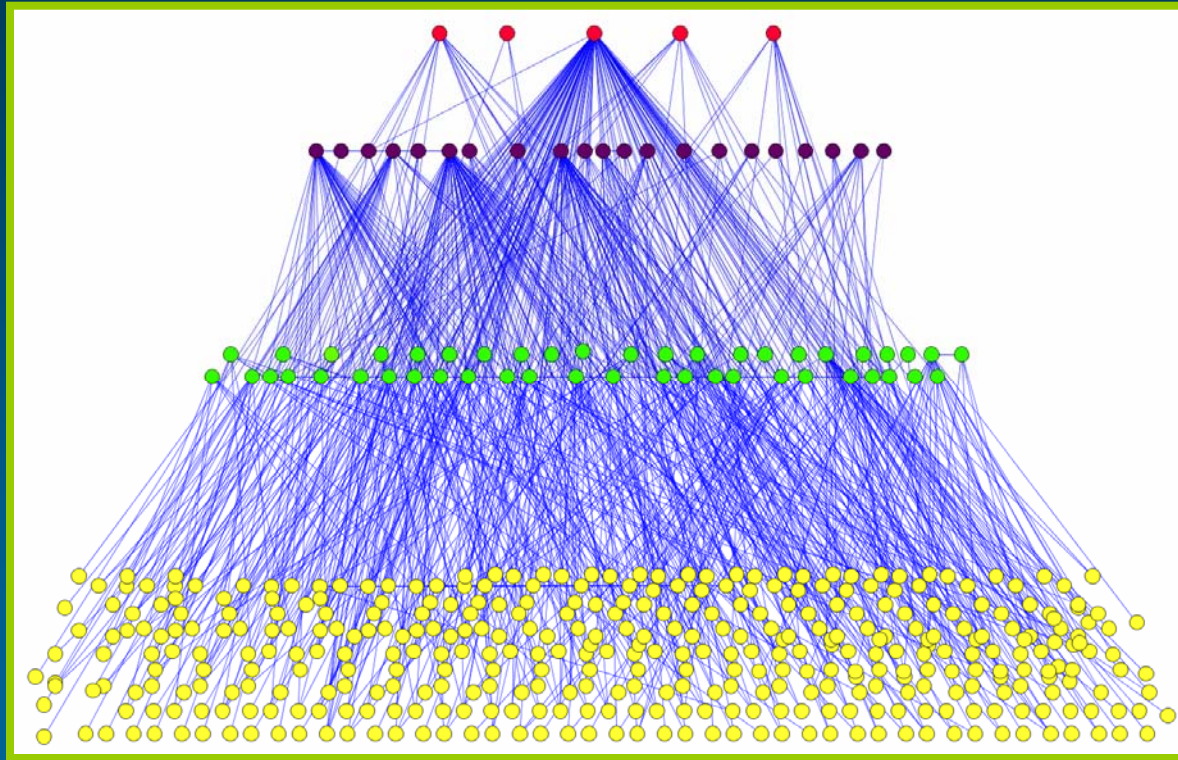
Non-glucose media Glucose media

R-Matrix Formalism

- quasi-stoichiometric formalism
- a structured and self-contained representation of Transcriptional Regulatory Network (TRN)
- can be quantitatively interrogated relying on the principles of the constraint-based modeling approach

Where is this going (short-term)?

E. coli Regulatory Network



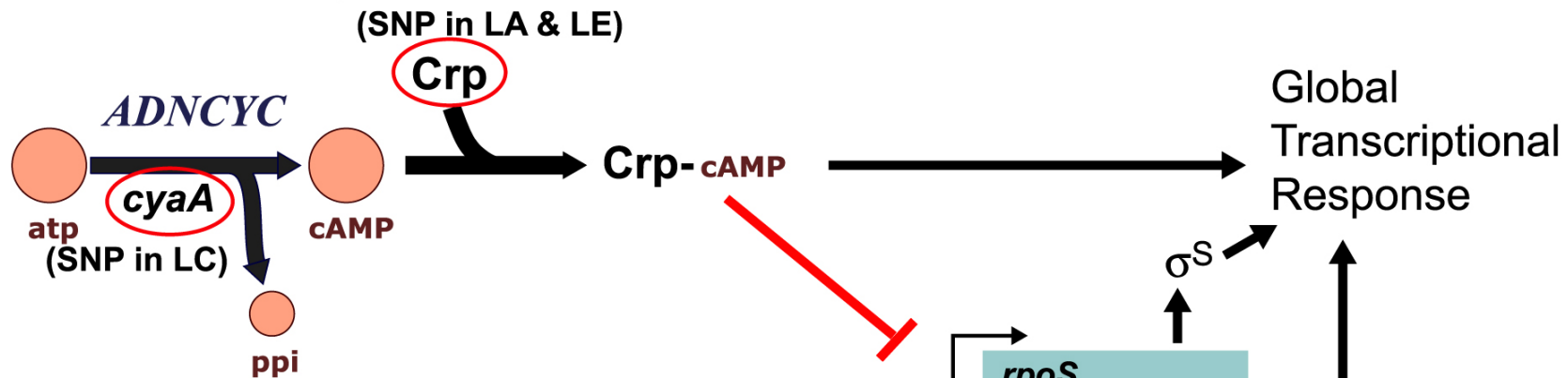
Reconstruction:

- 640 regulated genes
- 95 metabolites, 101 transcription factors, 26 reaction fluxes, 14 specific environmental factors
- 3 levels of transcription

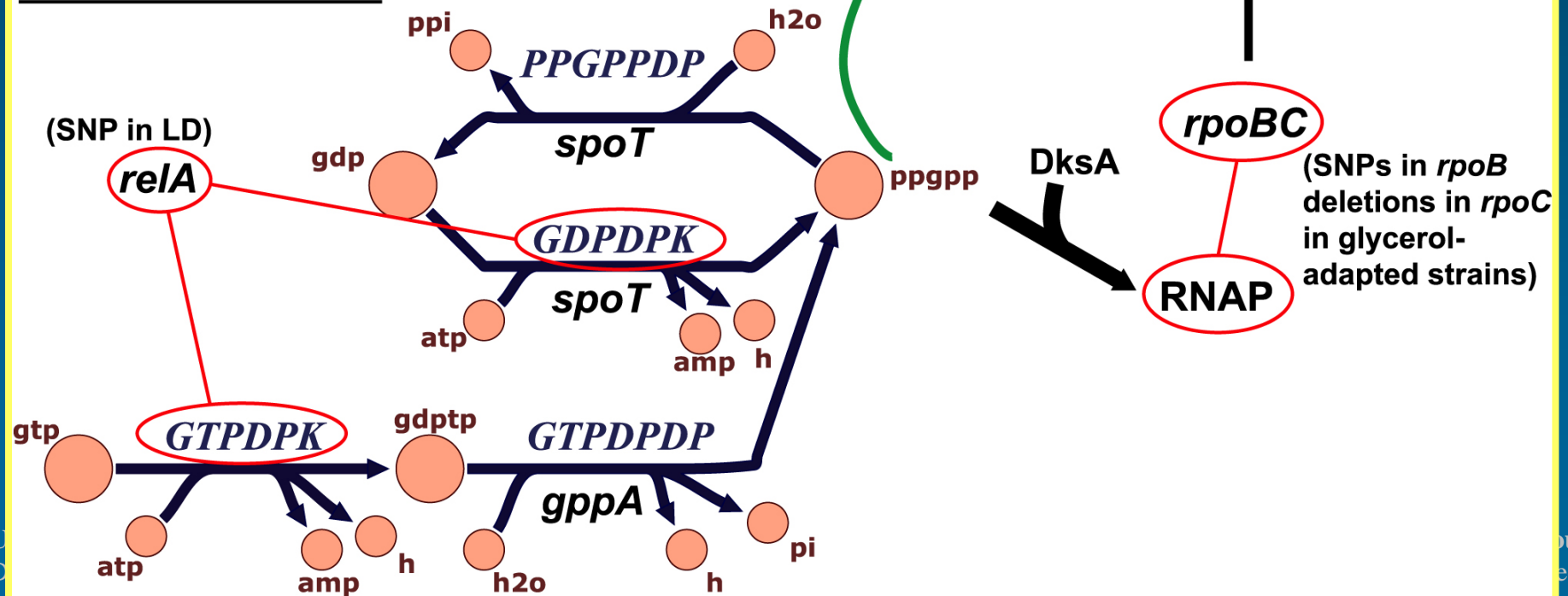
#5: mathematical formalisms are
developing that integrate
metabolic and TRN at the g/s

Global Regulatory Response

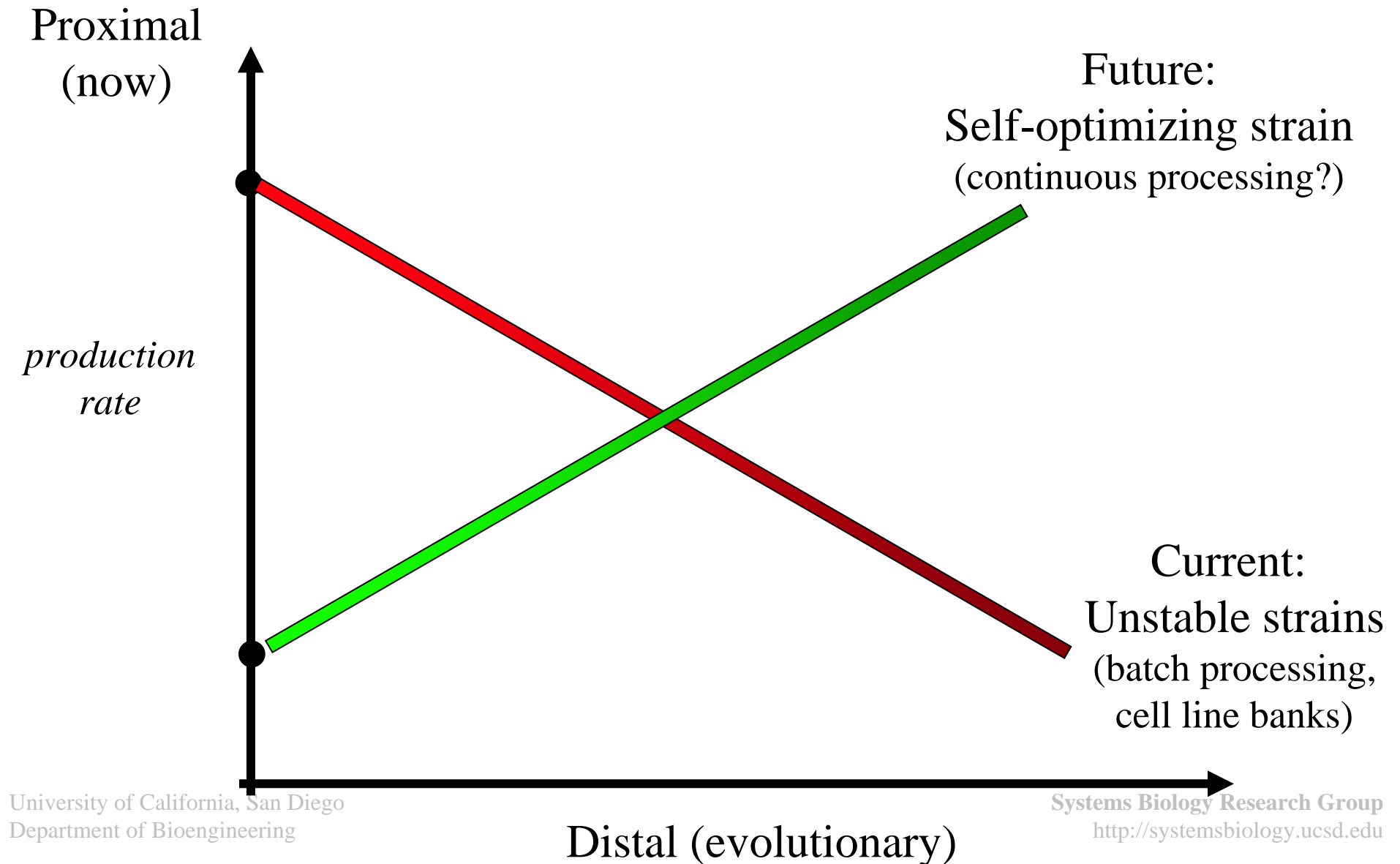
cAMP Production



ppGpp Biosynthesis



Causation in Biology: new approaches to strain design



#6: we can re-sequence to study
the genetic basis for TRN
adaptation at the g/s

Conclusions/issues

- Metabolic reconstruction methods established
- Conceptual framework for TRNs function at g/s in place
- Technology now available for direct measurement of TRN events on g/s
- 180 putative TF in E. coli -- expensive
- In silico methods to assemble, analyze the data are developing
- Integration with metabolism possible
- The genetic basis for adaptation can now be monitored